Supermarket Ginger Powder Increases the Mating Success of Mass-Reared Males of the Mediterranean Fruit Fly

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Abstract. A series of studies has shown that exposure to the aroma of ginger root oil (*Zingiber officinale*, GRO hereafter) enhances the mating success of mass-reared, sterile males of the Mediterranean fruit fly (medfly), *Ceratitis capitata*. The use of GRO to enhance male mating competitiveness appears to represent a simple means to increase the efficacy of the Sterile Insect Technique (SIT) against this pest. However, the relatively high cost of GRO, along with its restricted commercial availability, may preclude its use in SIT programs with limited budgets and/or no domestic supply of the oil. Consequently, we assessed the effectiveness of supermarket-brand, ginger powder, as a potentially less expensive and more widely available substitute for GRO, by comparing the mating success of powder-exposed (treated) and non-exposed (control) sterile males in competition against males from a recently established colony for matings with females from this same colony. In one set of experiments, sterile males were exposed to 2 or 10 g of the ginger powder placed inside storage boxes. In a second set, 1 or 2 g of powder was added to the sugar-agar food block placed on the storage boxes, and the food block was applied on the same day as pupal placement or on the day of peak adult emergence (2 d later). Treated sterile males achieved a significantly greater proportion of total matings than control sterile males at both doses of ginger powder in both sets of experiments, except that ginger powder had no detectable effect (at either dose) when added to food blocks placed on boxes on the same day as pupal placement. The cost of applying GRO to individual storage boxes is compared with that of ginger powder.

Key words: *Ceratitis capitata*, Mediterranean fruit fly, sterile insect technique, ginger powder, mating success

Introduction

A series of studies (Shelly 2005 and references therein) has shown that exposure to the aroma of ginger root oil (*Zingiber officinale*, GRO hereafter) enhances the mating success of mass-reared, sterile males of the Mediterranean fruit fly (medfly), *Ceratitis capitata*. Although the mechanism is not known definitively, preliminary observations indicate that GRO aroma interacts with the male exoskeleton in some way to produce a scent attractive to females. As males from long-established colonies are often inferior sexual competitors to wild males (Lance et al. 2000), the use of GRO to enhance male mating competitiveness, a protocol termed 'aromatherapy" (Shelly et al. 2004), appears to represent a simple means to increase the efficacy of the Sterile Insect Technique (SIT) against this agricultural pest. In fact, the ongoing medfly control program in southern California incorporated aromatherapy as part of their standard operating procedures in early 2005 (M. War, personal communication).

Relative to other programmatic costs, purchase of GRO is a minor expense. Nonetheless, the relatively high cost of GRO ($68 per kg for purchase of 5 kg, L. Milack, personal communication), along with its restricted commercial availability, may hinder the implementation of aromatherapy in SIT programs with limited budgets and/or no domestic supply of the

oil. To circumvent this problem, we assessed the effectiveness of supermarket-brand, ginger powder, as a potentially less expensive and more widely available substitute for GRO, in boosting the mating success of sterile male medflies.

Materials and Methods

Methods used to maintain *C. capitata* and conduct the mating trials follow Shelly et al. (2004), which should be consulted for additional details.

Study insects. Owing to the limited availability of wild flies, we used flies from a recently established colony (hereafter referred to as REC flies) started with > 1000 adults reared from coffee berries collected on Kauai. Eggs from this colony were placed on larval medium over vermiculite, which provided a pupation substrate for mature larvae. Adults used in the mating tests were separated by sex before reaching sexual maturity and maintained on a sugar-protein (yeast hydrolysate) mixture (3:1 ratio, v/v) and water. When used, REC flies were 7-12 d old and 7 generations removed from the wild.

Mass-reared flies were from a genetic sexing strain (Vienna7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu. This strain contains a temperature sensitive lethal [*tsl*] mutation, which allows culling of female embryos and exclusive production of males (Franz et al. 1996). Pupae were coated with fluorescent pink dye, packed into plastic bags, and irradiated (150 Gy with a ^{137}Cs source) 2 d before eclosion. After irradiation, pupae were placed in 6 paper bags (100 ml of pupae per bag; 1 ml contains ≈ 60 pupae), which, in turn, were placed inside individual storage (PARC) boxes (60 x 48 x 33 cm, l:w:h). Most adult emergence occurred 2 d after pupal placement, and emerging *tsl* males, which walked or flew out of the open paper bags, were fed a sugar-agar gel placed on a screened opening on top of the box. Both REC and *tsl* flies were maintained at 23-27 °C, 50-90% RH, and a photoperiod of 12:12 (L:D) h.

Exposure protocol. We exposed *tsl* males to ginger powder (yielding treated males) either directly or as a dietary ingredient. In the first case, we placed 2 or 10 g of ginger powder (McCormicks®) in a Petri dish, covered it with nylon screening (to prevent fly contact with the powder), and then quickly placed the Petri dish on the floor of a storage box (through a cork-capped hole drilled into a side of the box). The powder was applied between 0800-0900 hrs on the third day following peak emergence and left in place until flies were removed for testing. At most, several dozen flies escaped when the powder was introduced, and the loss of this small number of flies was assumed to have no effect on the holding environment inside the box. For each storage box set up with ginger powder, we also set up a storage box that received the same amount of pupae and sugar-agar gel but had no ginger powder placed inside, thus yielding nonexposed (control) *tsl* males.

In the second method, we added 1 or 2 g of ginger powder to the sugar-agar gel placed on a single storage box. The recipe for the gel was the same as that used in the SIT program in California (% by weight): water 84.52, sugar 14.71, agar 0.76, and methyl paraben 0.01. The powder was added after heating the mixture, when the gel was cooling but still fluid (65–75°C). Following the protocol of the California program, a 20 x 15 x 3 cm (l:w:h) slab of sugar-agar gel was supplied to individual storage boxes. For all boxes, the sugar-agar gel was prepared in a baking pan 1 d before use, covered with aluminum foil upon cooling, and stored overnight in a refrigerator (12–14°C). As above, for each storage box set up with powder-containing sugar-agar gel, we also set up a storage box that received the same amount of pupae and sugar-agar gel without ginger powder, thus yielding nonexposed (control) *tsl* males. In one set of mating trials, the sugar-agar gel (both standard and ginger powder-supplemented) was placed on the storage boxes on the same day the pupae were

placed in the boxes. In a subsequent set of trials, the sugar-agar gel was not placed on the boxes until the day of peak adult emergence (i.e., 2 d after pupal placement). In both sets of trials, the sugar-agar gel was left in place until males were removed for testing.

For both exposure protocols—directly as a powder or indirectly as an ingredient in the sugar-agar gel—we set up 4 storage boxes (2 treated, 2 control) on a given day, and *tsl* males from a given box were used in only 1 field-tent (i.e., 4 tents were conducted per test day, see below). Boxes receiving ginger powder were kept in a separate room from those not receiving the powder to prevent inadvertent exposure of control *tsl* males. Ginger powder was never provided to REC males in this study.

For both treated and control storage boxes, *tsl* males were removed for testing 4 d after peak emergence. For a given box, we removed one paper bag, quickly transferred it to a screen cage, and gently shook it to disperse the males. Approximately 100 males were then aspirated to screen-covered, plastic buckets and held for testing 1 d later (i.e., the majority of *tsl* males were 5 d old when tested). Both treated and control males were given standard sugar-agar gel following their removal from the storage boxes.

Mating trials. Mating trials were conducted at the USDA-ARS facility in Honolulu, Hawaii. Groups of 75 REC males, 75 REC females, and either 75 treated *tsl* males or 75 control *tsl* males were released into nylon-mesh, field cages (diameter 3 m, height 2.5 m) containing 2 artificial trees (*Ficus benjamina* type) at 0800 hrs, and all mating pairs that were observed were collected over the next 4 h. Male identity was later scored using a UV black light (dyed = *tsl* males, non-dyed = REC males). For all experiments, tests were performed over 6 different days (2 treated and 2 control trials per day) for a total of 12 replicates for both treated and control males, respectively.

Statistical analyses. As the assumptions of normality and equal variance were met in all cases, we compared the numbers of matings obtained by REC and *tsl* males for a given experiment using the Students t-test. We based between-experiment comparisons on relative mating success (proportions of total matings) using t-tests as well, with data arcsine transformed to increase normality. Sample size was 12 for all test groups, hence df = 22 for all t-tests.

Results

Ginger powder placed in storage boxes. The introduction of ginger powder to storage boxes increased the mating competitiveness of *tsl* males for the two doses tested (Table 1). For the 2 g dose, REC males were competitively superior to both control and treated *tsl* males, but treated *tsl* males obtained a significantly higher proportion of the total matings than did control *tsl* males (29 versus 13%, respectively, $t = 2.6$, $P < 0.05$). For the 10 g dose, REC males obtained significantly more matings than control *tsl* males, but there was no significant difference in mating success between REC males and treated *tsl* males. Correspondingly, treated *tsl* males achieved a significantly higher proportion of the total matings than did control males (43 versus 19%, respectively, $t = 4.4$, $P < 0.001$). In addition, *tsl* males exposed to 10 g of ginger powder achieved a significantly higher proportion of the total matings than did *tsl* males exposed to only 2 g of the powder (43 versus 29%, respectively, $t = 2.2$, $P < 0.05$).

Ginger powder as an ingredient in sugar-agar gel. When added to the food gel, ginger powder had no effect on the mating success of *tsl* males when the sugar-agar gel was placed on the storage boxes on the day of pupal placement (Table 2). For both doses of ginger powder tested, REC males obtained significantly more matings than either control or treated *tsl* males, and there was no difference in the proportion of total matings achieved by treated and control *tsl* males at a dose of 1 g ginger powder (24 versus 28%, respectively; $t = 0.4$,

Table 1. Results of mating trials in which treated tsl males were exposed to ginger powder placed inside storage boxes.

Ginger powder (g)	Matings per male type	% Matings per replicate	t	*tsl* males
2	REC	33.1 (2.0)	11.6***	
	Control *tsl*	5.5 (1.3)		13
	REC	28.7 (2.6)	4.6***	
	Treated *tsl*	11.8 (2.5)		29
10	REC	30.4 (1.7)	11.2***	
	Control *tsl*	7.3 (1.2)		19
	REC	22.4 (2.1)	1.9^{NS}	
	Treated *tsl*	16.9 (1.8)		43

Values for matings represent means (± SE) of 12 replicates. Numbers of matings obtained by REC and *tsl* males were compared with the t-test; significance levels were designated as: *** $P < 0.001$, NS not significant.

Table 2. Results of mating trials in which treated tsl males were exposed to ginger powder as an ingredient of the sugar-agar gel.

Ginger powder (g)	Matings per male type	% Matings per replicate	t	*tsl* males
A. Food placed on day of pupal placement				
1	REC	31.5 (2.3)	7.5***	
	Control *tsl*	9.8 (1.1)		24
	REC	28.5 (2.9)	6.6***	
	Treated *tsl*	11.2 (1.4)		28
2	REC	36.6 (2.8)	8.2***	
	Control *tsl*	13.1 (1.7)		26
	REC	32.8 (2.4)	7.7***	
	Treated *tsl*	15.7 (1.5)		32
B. Food placed on day of peak adult emergence (2 d after pupal placement)				
1	REC	29.5 (2.3)	8.8***	
	Control *tsl*	6.5 (1.2)		18
	REC	22.9 (1.3)	3.6**	
	Treated *tsl*	15.8 (1.4)		41
2	REC	34.4 (2.2)	10.5***	
	Control *tsl*	8.7 (1.0)		20
	REC	25.0 (1.8)	2.0^{NS}	
	Treated *tsl*	19.8 (1.9)		44

Values for matings represent means (± SE) of 12 replicates. REC and *tsl* males were compared with the t-test; significance levels were designated as: *** $P < 0.001$, ** $P < 0.05$, ^{NS}not significant.

$P > 0.05$) or a dose of 2 g ginger powder (26 versus 32%, respectively; $t = 0.7$, $P > 0.05$). In addition, there was no difference in the relative mating success of *tsl* males exposed to 1 versus 2 g of ginger powder (28 versus 32%, respectively; $t = 0.5$, $P > 0.05$).

In contrast, ginger powder had a significant effect on the mating success of *tsl* males when the sugar-agar gel was placed on the storage boxes on the day of peak adult emergence (Table 2). For the 1 g dose, REC males were competitively superior to both control and treated *tsl* males, but treated *tsl* males obtained a significantly higher proportion of the total matings than did control *tsl* males (41 versus 18%, respectively, $t = 4.6$, $P < 0.001$). For the 2 g dose, REC males obtained significantly more matings than control *tsl* males, but there was no significant difference in mating success between REC males and treated *tsl* males. Correspondingly, treated *tsl* males achieved a significantly higher proportion of the total matings than did control males (44 versus 20%, respectively, $t = 6.1$, $P < 0.001$). Among treated *tsl* males, there was no evident dose-dependent effect: *tsl* males exposed to 1 g of ginger powder in the food block achieved a similar proportion of the total matings as *tsl* males exposed to 2 g of the powder (41 versus 44%, respectively, $t = 1.1$, $P > 0.05$).

Discussion

Results of the mating trials were similar for sterile males of *C. capitata* exposed to ginger powder (1) placed within the storage boxes and (2) mixed in sugar-agar food blocks placed, in turn, on the storage boxes on the day of peak emergence. In both cases, when competing with REC males, the treated, sterile males achieved a significantly greater proportion of total matings than control, sterile males at the two doses tested. Moreover, at the higher doses used in the respective experiments (10 g powder placed within the boxes and 2 g powder mixed with the sugar-agar block), sterile males had a similar mating frequency as REC males. In contrast, ginger powder had no detectable effect on the mating success of sterile males (at either dose tested) when added to food blocks placed on boxes on the same day as pupal placement. In these latter trials, control and treated sterile males accounted for 24–32% of the total matings, and there was no statistical difference in their performance. Earlier work (Shelly 2001) showed that exposing late-stage pupae (i.e., 2 d before eclosion) to GRO had no effect on the mating success of subsequently emerged, adult males. Consequently, the ineffectiveness of the ginger powder in the present study most likely reflected the volatilization (and dissipation) of the ginger aroma prior to adult emergence.

At least at the higher doses tested, ginger powder appeared to be as effective as GRO in boosting the mating competitiveness of sterile *C. capitata* males. In the present study, sterile males exposed to 10 g of ginger powder directly or 2 g of ginger powder in the sugar-agar had a mating success equivalent to that of REC. Likewise, Shelly et al. (2004) found that application of 0.125–1.0 ml of GRO to individual storage boxes increased the relative mating success of sterile males from about 25% of total matings (control, non-exposed males) to approximately 50% (i.e., equivalent to that of REC males).

Although additional tests might identify lower effective doses of ginger powder, the present data at least allow preliminary cost comparisons between ginger powder and GRO. The cost of using GRO is \$0.0085–\$0.068 per storage box for doses of 0.125-1.0 ml GRO per storage box (at \$68 per kg of GRO, current price paid by California medfly SIT program, L. Milack, personal communication; the specific gravity of GRO is slightly below 1 but, for simplification, is here assumed to be unity). Based on a survey of on-line prices of various spice distributors, the cost of ginger powder is typically \$3.64/kg. For the placement of 10 g of ginger powder within storage boxes, the cost per box would be about \$0.036, or about half of the upper estimate for GRO. When mixed with the sugar-agar diet at a dose of 2 g (and applied on the day of peak emergence), the price per storage box would

be approximately $0.007, a value similar to, but slightly lower than, the lower estimate for GRO. These comparisons suggest that, for the exposure protocols tested, ginger powder and GRO entail similar costs, with the greatest difference noted between ginger powder as a dietary ingredient and the highest dose of GRO ($0.007 versus $0.068, respectively). Accordingly, the choice between ginger powder and GRO may depend more on the ease of purchase (domestically versus internationally) and associated shipping costs than on the cost per effective dose.

In conclusion, recent work (TES, unpublished data) has shown that placement of GRO, not on individual storage boxes, but at several locations inside holding rooms containing many storage boxes increases the mating competitiveness of the sterile males. Specifically, a total dose of 36 ml GRO (4 locations, 9 ml per location) results in a significant increase in mating success when placed in trailers holding approximately 360 storage boxes (or approximately 13 million males), resulting in an estimated cost of $0.007 per storage box (or $0.19 per million males). Whether or not ginger powder, when distributed among several locations in a holding room, can similarly boost male mating success is not known. From an economic standpoint, this information would be useful, because even an excessively large amount of ginger powder, such as 0.5 kg per trailer, would be less expensive than GRO ($0.005 per storage box or $0.14 per million males).

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